Hunterian Lecture

The genetic basis of severe osteoarthritis

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Osteoarthritis is the commonest disease of human joints.¹ It is a group of overlapping distinct diseases, which may have different aetiologies but with similar biological, morphological and clinical outcomes. The disease process not only affects the articular cartilage, but also involves the entire joint including the subchondral bone, ligaments, capsule, synovial membrane and peri-articular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration and full-thickness loss of the joint surface.²

Diseases such as idiopathic osteoarthritis have both environmental and genetic causes. They are known as multifactorial disorders and may be defined as being likely to affect more than 1% of the population at some stage in life. There is considerable interest in the genetic contribution to these common diseases because of their impact on mortality and morbidity in the industrialised world. Molecular biology has made it possible to define the genetic basis not only of single gene disorders but also of a number of more common disorders where genes and environment both play a part. Genes encode the structure of individual peptide chains from which all proteins are built. These proteins interact in the biological pathways that form the basis of metabolic processes. Abnormalities of these metabolic processes are ultimately due to abnormalities of genes.

Osteoarthritis has traditionally been regarded as an age-related condition or a wear-and-tear phenomenon with a large environmental component in its aetiology. In the genetic investigation of diseases such as osteoarthritis, it is important to establish what the relative contribution of genes is to the condition. In the 1940s, Stecher studied

nearly 70,000 American adults and found the prevalence of osteoarthritis in mothers and sisters of affected white females to be 2–3 times those expected.³ It was the studies from Leigh and Wensleydale in the north of England undertaken by Kellgren and Lawrence in the 1950s that first revealed a familial basis to hip osteoarthritis. They conducted a radiographic survey of the first-degree relatives of those individuals with radiological evidence of osteoarthritis.⁴ The prevalence of osteoarthritis in these relatives was compared with the general population and was more than twice the expected rate. Twin studies form the classic basis of assessing heritability. Spector and colleagues in 1996 reported a significantly greater concordance in identical middle-age female twins than non-identical twins. They obtained heritability estimates of 39–65% for the combined presence of radiographic hand and knee osteoarthritis.⁵

Relative risk and heritability

Another method of assessing the genetic component of disease is to calculate the relative risk. This is a quantification of the risk of the disease recurrence to any relative of a proband. It is really a comparison of the incidence of the disease in specific relatives of probands compared to the incidence of the disease in the general population. Generally, values of between 1.5 and 4.5 exist in common disorders with a genetic basis such as peptic ulceration or breast cancer. In disorders such as osteoarthritis, this calculation is difficult because the disease occurs later in life and there needs to be some correction factor to take account of this.

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Between August 1995 and April 1996, medical records and radiographs of all patients who underwent a primary or revision hip or knee replacement in Oxford were reviewed. Only patients undergoing primary replacements for idiopathic osteoarthritis were included. Non-Caucasian patients were also excluded. Patients selected for study were invited to participate in a short questionnaire. Enquiry focused on the age, gender and history of any hip or knee replacement for idiopathic osteoarthritis in surviving and deceased full siblings and spouses of the patients. The relative risk and heritability estimates for symptomatic osteoarthritis of the hip and knee, therefore, used hip and knee replacement as indicators of severe symptomatic osteoarthritis.

Risk estimates were based on joint replacement history of siblings and spouses of index cases and were obtained using the equation:

$$\lambda_R = \frac{\text{Proportion of relatives of specified type affected by disease}}{\text{Proportion of population affected by disease}}$$

Heritability estimates were calculated by comparing the liability to total joint replacement in siblings with that in the general population as given by Falconer in 1989.⁶ It is assumed that the liability to osteoarthritis is distributed normally for both siblings and spouses.

From a consecutive series of 721 total hip or knee replacement patients, 319 cases were excluded. Of the remaining 402 patients who participated in the study, 393 were seen and 9 were contacted by telephone. Overall, 256 patients have undergone unilateral hip replacement, 112 unilateral knee replacements, 8 bilateral hip replacements, 24 bilateral knee replacements and 2 ipsilateral hip and contralateral knee replacements. Of these, 337 operations were primary replacements and 65 were revisions. Table 1 describes the relative risks and heritability estimates.⁷

This is the first study to investigate relative risk and heritability in end-stage osteoarthritis of the hip and knee and clearly demonstrates that there is a familial basis to the disease manifesting in this way. It may seem that these relative risks are small. However, in diseases as common

as osteoarthritis, Weiss has calculated that even in cases of a hypothetical autosomal dominant disorder siblings will only have a 5-fold increase relative to the general population, assuming that 10% of the population are independently affected.⁸

Genome-wide search

Encouraged by the importance of discovering more about the biological basis of osteoarthritis, we pursued an investigation into susceptibility genes for osteoarthritis. As with single gene disorders, common multifactorial diseases also aggregate in families. However, the proportion of affected first degree relatives of probands in these diseases is only around 5% greater than the prevalence in the general population, much less than that seen with autosomal dominant disorders. The genetic component of such disorders is thought to be polygenic resulting from the interaction of several genes in different parts of the genome. Although much commoner than single gene disorders, multifactorial diseases are often much less striking in presentation and appear later in life. Investigation of the genetic components of these disorders is made difficult by long association with an environmental component, which may also be poorly understood.

We were, therefore, faced with the difficulty of trying to design a genetic study that would allow us to dissect out the genetic component of this clearly very complicated disease. Recent genetic analyses have used the presence of subtle inherited differences in the DNA of different individuals. Enzymes (restriction endonucleases) capable of selectively dividing DNA at specific sites have been discovered. DNA from different individuals exposed to the same endonucleases can be divided into different lengths using these enzymes. The lengths of DNA produced are known as restriction fragment length polymorphisms. Genetic analysis using these restriction fragment length polymorphisms in sporadic families with premature idiopathic osteoarthritis have been conducted. Studies in Finish families have also described abnormalities of type II collagen in large pedigrees with osteoarthritis. These families probably had a mild skeletal dysplasia rather than idiopathic osteoarthritis.⁹ One of the

Table 1 Relative risks and heritability estimates for total joint replacement of the hip (THR) or the knee (TKR) or both (TJR) for idiopathic osteoarthritis in siblings as compared to spouses of index cases

Total joint replacement	Number of probands	Relative risks to all siblings and spouses (95% CI)	Relative risks to siblings and spouses over 64 years (95% CI)	Heritability (h ²)
TJR	402	1.98 (1.11–3.51)	2.32 (1.22–3.69)	31% (all relatives)
THR	281	1.78 (0.92–3.45)	1.86 (0.93–3.69)	27% (all relatives)
TKR	121	4.8 (0.64–36.4)	Insufficient numbers	Insufficient numbers

CI, confidence interval.

problems in investigating idiopathic osteoarthritis is the late onset of the disease and classical linkage studies are impossible. Therefore, we decided to use a method relying on affected sibling pairs alone. The basis of this is that siblings can be expected to inherit the four alleles of their parents in a predictable manner. Across a population of siblings, 25% will inherit the same alleles, 50% will share one allele and 25% will share neither allele. If one studies siblings that share the same abnormality, for example osteoarthritis of the hip or knee, then it is expected that a random marker located close to the gene responsible for osteoarthritis of the hip or knee would be skewed with regard to the sharing of alleles. A number of candidate genes have been studied as potential susceptibility loci for idiopathic osteoarthritis but the results are conflicting.^{9,10} This may simply be a reflection of the complexity of the disease. Overall, therefore, we felt that the genetic dissection of idiopathic osteoarthritis merited a systematic genome screen using anonymous polymorphic microsatellite markers. We recruited sibling pairs using joint replacement surgery resulting from idiopathic osteoarthritis as our ascertainment criteria. Our aim was to use families whose idiopathic osteoarthritis was severe and, therefore, more likely to have a genetic component. We used a two-stage approach similar to that proposed by Holmans and Craddock in 1997.¹¹ In stage I, we genotyped 272 microsatellite markers in 297 of 481 families. In stage II, microsatellites that demonstrated evidence for linkage at a nominal P less than 0.5 in these 297 families were then analysed in the remaining 184 families. This two-staged approach demonstrated linkage of markers.

Patients and Methods

Families with at least two siblings each of which had undergone one or more replacement of the hip, knee or both for primary idiopathic osteoarthritis were recruited. Overall, 719 individuals had undergone only total hip replacement and 198 had undergone only total knee replacement; 66 individuals had undergone hip and knee replacements. Heberden's nodes were present in 38.5% of the affected individuals. The majority (58.3%) of these individuals had at most three nodes. The collection of the families was undertaken through the records of the Nuffield Orthopaedic Centre in Oxford, the Musgrave Park Hospital, Belfast and the Wishbone Trust Charity. The Wishbone Trust Charity generously allowed contact through participants in 'The Great Hip and Knee Walk'. This allowed us to contact over 40,000 individuals who had undergone either hip or knee replacements. The patients were all interviewed by trained research nurses. Radiographs and, when available, histological samples were reviewed. We excluded all cases other than primary

idiopathic osteoarthritis. Of the 481 families recruited, only three had a parent who was able to participate. Therefore, we collected additional siblings who had not undergone hip or knee replacement to assist in the determination of identical by descent allele transmittance. The 481 families comprised 1054 affected individuals plus 302 additional siblings. Of the affected individuals, 625 (59.3%) were women and 429 (40.7%) were men. The average age of the affected individuals at the time of their first operation was 66 years (SD, 9.0 years) with an average of 66 years (SD, 9.3 years) in affected women and average age of 65 years (SD, 8.6 years) in affected men. From each individual 25 ml of venous blood was collected into EDTA tubes and DNA was extracted by conventional techniques.

The initial screening panel included 292 microsatellite markers. This is the panel described by Read *et al.* in 1994.¹² Additional markers were used to provide coverage of chromosome 11 from the genome database or from the prism linkage map set.

Parents were rarely available so extensive error checking procedures were employed for all families and for each marker. Markers considered to be unreliable were eliminated from the study. The linkage analysis strategy was in two stages, the first involving 297 of our 481 families. Any marker that had a nominal P value of less than 0.5 in stage I would then be examined in the remaining 184 families (stage II). The aim of this strategy was to take through to stage II only those markers that demonstrated reasonable evidence of linkage. If a marker's P value for stages I and II combined was no more than the P value for stage I, then it would support linkage at that marker.

We stratified by sex and joint replaced (hip or knee). We adjusted lod scores and P values to correct for the 6 strata tested (women only, men only, hips only, knees only, female hip and male hip).

Results

Sixteen markers from stage I showed evidence of linkage. These markers were then genotyped in the remaining 184 families of stage II. The results are shown in the Figure 1. Three of the four markers were on chromosome 11. Most of the chromosome 11 markers from stage I were not taken through to Stage II because they did not reach the necessary threshold levels. Many epidemiological studies have shown that there is a preponderance of osteoarthritis in women. This may be due to different environmental exposures in the two sexes. However, a Finnish study in 1996¹³ suggested that genetic susceptibility may be greater in women than in men and this result has been supported by segregation analysis performed by Felson

Figure 1 Multipoint analysis of chromosome 11, for stages I and II combined (from Chapman *et al.*¹⁵).

in 1998.¹⁴ Not only have differences in heritability between women and men been reported, it has also been suggested that there are heritability differences between groups

defined by type of affected joint. These differences may be the result of genetic locus heterogeneity. The stratification analysis revealed that the linkage to chromosome 11 was predominantly accounted for by the affected women-only pairs with a single lod point score of 2.54. The results are shown in Figure 2.¹⁵ We undertook further stratification and found further linkage localisations on chromosomes 2, 4, 6 and 16 and these are represented in the Figures 3 and 4.^{16,17} These further linkages occurred as a result of stratifying the whole genome screen.

Discussion

We have identified regions on chromosomes 2, 4, 6, 11, and 16 that are linked to severe symptomatic osteoarthritis of the hip and knee. Stratification has strengthened these linkages for hip arthritis in women. These linkages serve as a starting point for techniques that may further narrow the interval of linkage and allow cloning of the gene or genes that cause susceptibility to osteoarthritis.

Osteoarthritis is an extremely important disease. It is the main cause of joint replacement surgery and is so common that in the industrialised world it is set to become the fourth highest impact condition in women

Figure 2 Multipoint analysis of chromosome 11, for stages I and II combined, with data stratified. (A) Female only pairs ($n = 196$ families). (B) Total hip replacement pairs ($n = 311$). (C) Female total hip replacement pairs ($n = 132$). From Chapman *et al.*¹⁵

Figure 3 Multipoint analysis of chromosome 2q with the data stratified. (A) Female only pairs ($n = 196$ families). (B) Total hip replacement pairs ($n = 311$). (C) Female total hip replacement pairs ($n = 132$). From Loughlin *et al.*¹⁶

and the eighth most important in men.¹⁸ The majority of people with osteoarthritis are not severely affected and symptoms of pain from joints and the spine depend also on cultural and social factors. Another important feature is that most mild osteoarthritis does not progress to the severe disease requiring joint replacement surgery. Dieppe¹⁹ has suggested that because the condition is so common and yet most of it relatively benign, we should make severe disease that leads to considerable pain and disability a priority. It is for this reason that we have concentrated in our studies on investigating the genetic background to severe osteoarthritis of the hip and knee. It is worth remembering that articular cartilage is a complex tissue. It does not have any specific blood supply or nerve supply and, although its organisation is complex, it is composed of very few cells. Indeed, the underlying pathology in osteoarthritis may not be due to problems in the articular cartilage but may arise from problems in other parts of the joint such as the synovium or subchondral bone. Conventional investigation of severe osteoarthritis is fraught with difficulty and this makes an understanding of the genetic pathways at the basis of the condition very appealing.

Figure 4 Multipoint analysis. (A) Chromosome 4, female hip ($n = 85$ families). (B) Chromosome 6, hip only ($n = 194$ families). (C) Chromosome 16, female hip ($n = 85$ families) and female only ($n = 132$ families). From Loughlin *et al.*¹⁷

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